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Mini Review

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Measurement of free light chains with assays based on monoclonal antibodies

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Abstract: Recently, serum free light chain (FLC) assays incorporating anti-kappa (κ) and anti-lambda (λ) FLC monoclonal antibodies have become available: N Latex FLC assay (Siemens) and Seralite® (Abingdon Health). The purpose of this review is to provide an overview of these two new monoclonal antibody-based methods. In doing so, the review will outline the performance characteristics of each method, including a summary of: assay principles, antibody specificity, analytical performance and assay performance in disease. Additionally, the review will describe the potential user benefits of adopting these new generation FLC assays, which are designed to overcome the established limitations of existing polyclonal antibody based FLC assays.

Keywords: free light chains; myeloma; point of care; turbidimetry.

Introduction

The key components for any kappa (κ) and lambda (λ) free light chain (FLC) assay are the detection antibodies. These antibodies ultimately determine the performance characteristics of the assay in which they are incorporated. To be defined as effective antibodies, several factors must be considered.

Firstly, the antibodies can be either monoclonal or polyclonal. The use of monoclonal antibodies (mAbs) results in higher lot-to-lot consistencies in assay performances, and does not need renewed immunization, purification and processing to obtain specificity, as required for polyclonal antibodies. Clearly, a substantial benefit of reduced batch variation is the potential for more reliable patient monitoring, particularly when tracking patients through disease therapy and remission. Furthermore, most mAbs can be selected to have higher affinities than polyclonal antibodies and have better defined specificities, thus avoiding cross-reactivity with other proteins, including immunoglobulin bound-light chains (LC).

Both κLC and λLC are, by nature, highly heterogeneous molecules. The protein sequences show high variability in the antigen-binding domains. The constant (i.e. invariable domains) of the LC proteins are coded by only one gene for κ and up to seven genes for λ and therefore may serve as the best target for mAb binding to ensure full and consistent recognition of these proteins [1, 2]. Besides binding to the preserved sequences of the LC proteins, mAbs should bind to the exposed part of the LC protein that is hidden when LC is bound to immunoglobulin heavy chains.

Several studies have described the production of mAbs to FLCs that fulfill at least the criterion of binding to free and not to heavy chain-bound LC [2–5]. Using these mAbs, several assays have been developed, but not all of these tests have subsequently become commercially available.

In this review, two commercial methods are described which demonstrate that use of mAbs for the detection of FLC in serum is comparable with polyclonal-based assays for patient diagnosis and monitoring, and have the predicted analytical performance benefits of mAb-based assays. The N Latex FLC assays are nephelometric assays for the Siemens BN™-systems and the Seralite® assay is a rapid, lateral flow test.

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The N Latex FLC κ and λ assays

Assay principle

The N Latex FLC assays (Siemens Healthcare Diagnostic Products GmbH, Marburg, Germany) are latex-based

nephelometric assays for the measurement of κ and λFLC in serum, Li-heparin plasma, EDTA plasma, urine and cerebrospinal fluid [5]. The assays are specifically designed for the Siemens BN™ systems. For the assays, panels of specific murine mAbs to either κ or λFLC are covalently coupled to latex beads. With the use of more than one mAb in the assay, detection of all variations of LC proteins is supported. The latex beads ensure the needed sensitivity and reduce, together with the high affinity antibodies, the reaction time of the assays. Additionally, a strong detergent dissociates the sticky FLC molecules from hydrophobic surfaces and ensures recognition by the antibodies. Both FLC assays contain a short pre-reaction and a 10 min main reaction. The pre-reaction protects the reaction from antigen excess and prevents false negative results. The short pre-reaction is only possible with the use of high affinity antibodies. The pre-reaction starts immediately when a small amount of sample is added to the reaction cuvette and should give sufficient agglutination within the given pre-reaction time. During the agglutination reaction, non-specific reactions are prevented with the use of mouse immunoglobulins and strong detergents.

FLC antibody specificity

For the development of the FLC assays, panels of more than 150 monoclonal mouse antibodies were created. For this, mice were immunized with polyclonal human κ and λ from multiple donors. The mAbs were selected based on specificity in an ELISA system as previously described [5]. During the selection procedure more than 90% of the mAbs were found to be specific for the free κ and λ molecules and non-responsive to intact immunoglobulins. After cross matching, non-competing mAbs were tested on purified monoclonal and polyclonal κ and λFLC. The non-competing mAbs should recognize all allotypes of κFLC and isotypes of λFLC. The selected mAbs proved responsive to monoclonal and polyclonal FLC. In the next step the mAbs were covalently bound to latex beads and tested in the nephelometer. Once the final assay protocols on the BN™ systems were established, over 2150 clinical samples were tested for κFLC and λFLC in serum and compared to the Freelite® assays (Figure 1A and B) (unpublished data). There is a clear numerical difference between the methods especially for λFLC. With low and very high concentrations of FLC, the methods gave results which may differ more than 10-fold. At low concentrations, these differences are partially explained by 'the gap effect' in the Freelite® assays (for explanation see further on). This inaccuracy in the Freelite® assays strongly influences the ratio of involved/ uninvolved light chains. In Figure 1C, the direct comparison of the κFLC/λFLC ratio for both methods is shown. The overall identity for ratios identified as low, normal or increased was 86% with a Cohen's κ of 0.77. Within the reference ranges for the ratios, both methods show good similarity, while outside these ranges the ratios may show greater differences.

The constant domains of the light chains, as indicated by the name, are well preserved, but also contain a limited number of allotypes or isotypes [1]. The selected mAbs for the panels reacted with the constant domain of the light chains. The phenotype of monoclonal κ and λFLC may differ by a yet unknown mechanism and lead to substantial variations in the structure of the light chain molecule. Monoclonal κ and λFLC come in different shapes and sizes when the molecules multimerize [6–9]. The formation of these multimers may hamper or enhance recognition by the antibodies. The outcome of the measurement is based on the availability of the epitopes in the light chain molecule, more than the genetic variations in the constant domain of the FLC. Different sets of mAbs may therefore lead to different results, as shown during the development of the mAbs used in the Seralite® assay [2]. The spearman correlation between the results of different combinations of mAbs for λ was 0.97, but for κ the correlation was 0.78.

Analytical performance

The major advantages of the use of mAbs in the assays are the reduced variability between the lots, and the high affinity binding to the LC molecules. The lot-to-lot consistency of the assays was demonstrated to be 7.3% for κFLC and 10% for λFLC [5, 10, 11]. Pretorius et al. [10] and Lock et al. [11] tested only a limited number of lots, but when samples were measured on more combinations of calibrators and reagents these findings were confirmed (Figure 2). The N Latex FLC assays demonstrated a high lot-to-lot consistency, which allows for flexible calibration by combining different lots of latex reagents, supplementary reagent, standard and controls.

Good precision over the whole measuring range is of importance for calculation of the involved/uninvolved FLC ratio to accurately predict progression of smoulding multiple myeloma patients to active malignant disease [12]. The N Latex FLC assays showed good precision with %CVs <5%–7% over the whole measuring range, whereas Freelite® assays may have higher imprecision of up to 40% at very low concentrations [5, 10], probably due to dilution anomalies, that is, the so-called 'FLC gap

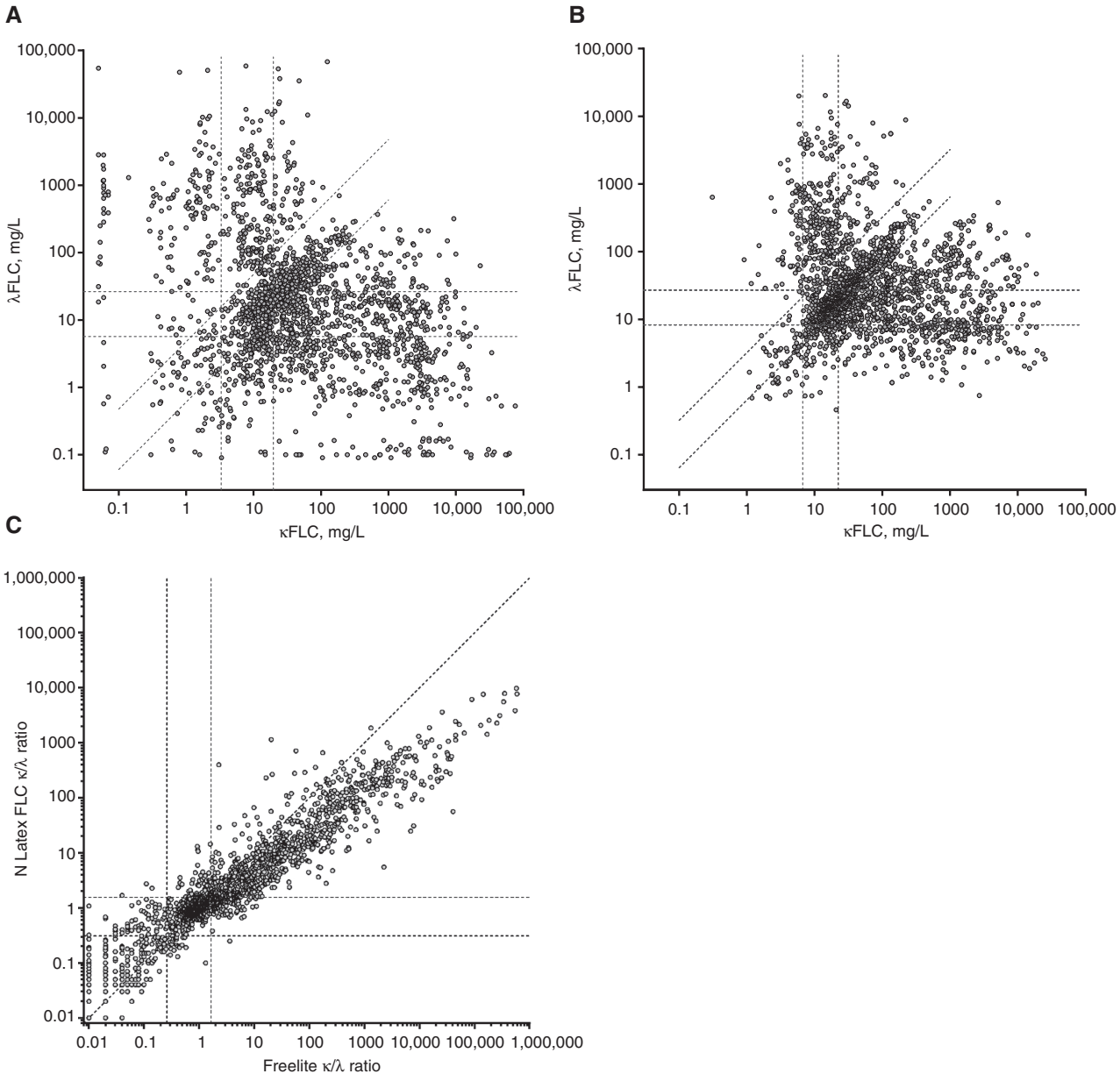

Figure 1: Parallel measurement of 2155 routine serum samples with the Freelite® (A) and the N Latex (B) κFLC and λFLC assays and the κFLC/λFLC ratio comparison between the methods (C).
In Figure A and B, the dotted horizontal and vertical lines indicate the reference ranges for the assays and the diagonal lines indicates the area with normal κFLC/λFLC ratios. In Figure C, the diagonal line indicates y=x. In this figure 144 samples are not included because either ratio was designed zero by the technician.

effect' [2]. The N Latex FLC assays do no suffer from the 'gap effect'.

The N Latex FLC assays show good linearity for κ and λFLC within the initial measuring dilution, but also for clinical samples with concentrations of κ and λFLC far above this range [5, 10, 13].

The N Latex FLC applications on the BN™ systems have built-in pre-reaction protocols to secure antigen excess protection. This protocol is highly effective for both κ and λFLC. However, certain monoclonal FLC samples show a non-linear dilution behavior, the result in the next dilution providing a higher result than expected [10, 14]. Jacobs et al. [14] studied the effect of sample dilution on recovery by N Latex FLC and Freelite® assays, and similar to the study by Vercammen et al. using Freelite® [15], showed that the detection of especially κFLC is significantly hindered by the presence of high concentrations of M-proteins [14, 15]. The detection of FLC in healthy donors

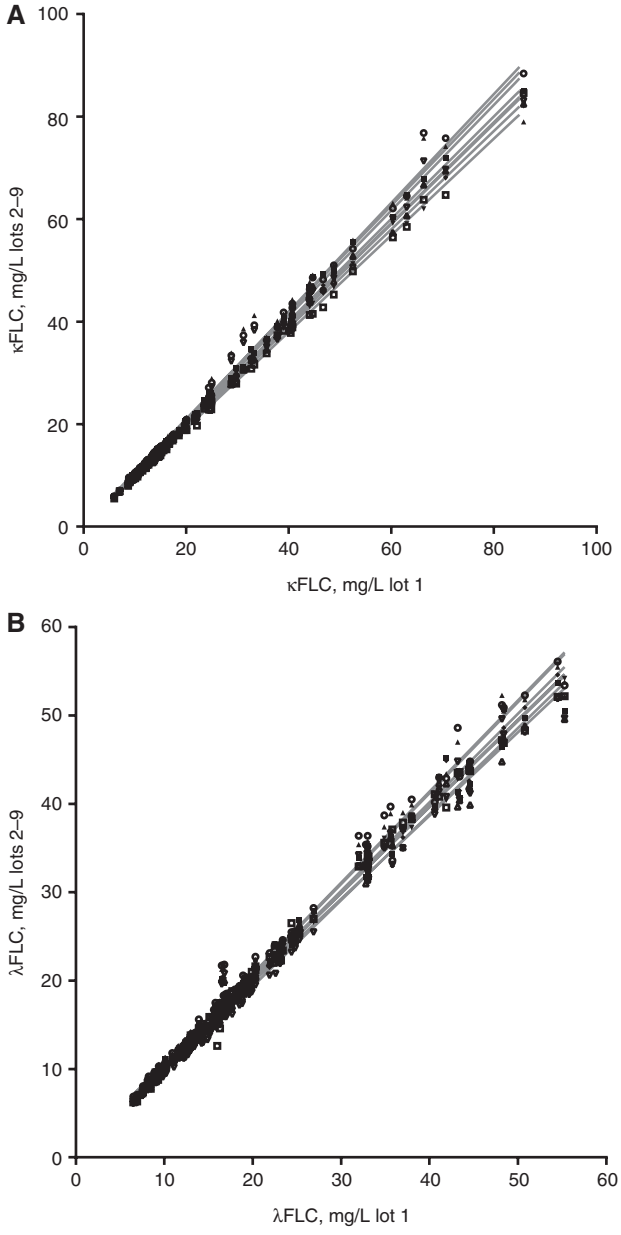

Figure 2: Lot to lot consistency of the N Latex κFLC (A) and λFLC (B) assays.
From: Ref. [5].

with at least 500-fold molar excess of bound LCs to heavy chains in immunoglobulins compared to free, unbound, LC gives no interference. When there is a high concentration of monoclonal intact immunoglobulin present, the ratio of bound versus FLC shifts to even higher numbers and the ratio bound versus free may easily exceed 5000-fold excess. There is always a chance that part of the monoclonal intact immunoglobulin is not correctly folded and part of the bound LC is exposed. If one epitope is exposed, part of the reagent is blocked by this bound LC, which results in false-low FLC concentrations in the initial dilution of the assay. The exposed epitope thus interferes with the reaction. The true concentration can only be given at higher dilutions of the samples. This interference is patient, that is, sample, specific and found with both Freelite® and N Latex FLC assays [14].

VanDuijn et al. described a new mass spectrometry (MS) analysis of the light chains in serum [16]. The correlations between the MS method, N Latex FLC assays and Freelite® were very good, but the MS method requires intensive processing before analysis because all bound LCs in intact immunoglobulins need to be discarded. The MS method also revealed gross overestimation by Freelite® measured on the Siemens BNII and some overestimation by the N Latex FLC. The difference between Freelite® and N Latex FLC in this context is remarkable, because the concentrations of the calibrators for the N Latex FLC are derived from one single lot of the Freelite® assays.

For the calibration of the N Latex FLC assays, the first master calibrator was made in 2008 in the same formulation as the calibrator and controls of the kit and stored at −80 °C. In 2008, this master calibrator was calibrated to the one lot of Freelite® assays. Due to the significant variability of Freelite® kits [17], the in-house master calibrator showed unacceptable high variability when measured on successive lots of Freelite®. All working standards are derived from this one-time calibrated lot of master calibrator.

The master calibrator is controlled each year for drift by measuring fresh samples from 200 donors from the blood bank population. The samples come from the same donor population that was used to establish reference ranges for these assays.

ANOVA gauge repeatability and reproducibility measurements were performed to determine the total amount of variability of the BN™ system itself. They were compared to the variability of the FLC measurement itself. The measurements were performed on 3 BN™ systems with 1130 κFLC and 920 λFLC results. The variation for κFLC was 4.4% and for λFLC was 3.1% (unpublished data).

Reference intervals were created with 253 EDTA plasma samples and 114 serum samples from normal healthy blood bank donors aged 18–70 years: κFLC 6.7–22.4 mg/L, λFLC 8.3–27 mg/L and FLC ratio 0.31–1.56 [5]. Jacobs et al. and Tate et al. studied patients with renal impairment with chronic kidney disease (CKD) class 1–5 [18, 19]. As κ and λFLC increased significantly but proportionally with every CKD increment, the N Latex FLC does not need special reference ranges for the FLC ratio in patients with renal impairment.

Assay performance in disease

The international guidelines indicate that the measurements of FLC should be combined with a clinical history and other diagnostic tests to reach high clinical sensitivity and specificity for different disease states [12, 17]. Just like the Freelite® assays, the N Latex FLC assays show diagnostic and clinical limitations partly due to the nature of the FLC proteins which may lead to reduced recognition [11, 20–22] Therefore FLC assays should be used in conjunction with other laboratory tests [13, 20]. In the diagnosis of AL amyloidosis, when the FLC measurement is combined with serum and urine protein electrophoresis and immunofixation (IFE) the diagnostic sensitivity, that is, the identification of the amyloidogenic clone, is reported to be 98% for both N Latex and Freelite® assays [23, 24].

When used in daily practice, the correlation and concordance rate for screening of patients with plasma cell dyscrasias between Freelite®, serum-IFE and N Latex FLC was good [8, 11, 13, 16, 21, 22, 25]. Although the studies were performed with limited numbers of patients, the N Latex FLC showed 97%–100% clinical specificity (true negative) and 60%–65% sensitivity (true positive), compared to 94%–97% and 61%–72% for Freelite®, respectively, when compared to serum-IFE [21, 22].

The guidelines for monitoring disease states are based on the Freelite® assays on the Siemens BNII system [17]. Numerical differences in the FLC ratio between the assays, partly due to the 'gap effect' in the Freelite® assays and to differences in λFLC concentration between N Latex and Freelite® assays mean that guideline response criteria based on Freelite® assays may not fully apply to the N Latex FLC assays [10, 17, 26–33]. However, N Latex FLC can accurately be used for prognostic stratification in AL amyloidosis patients based on the guidelines by Comenzo et al., as demonstrated by Mollee et al. and Palladini et al. [23, 24, 34]. The survival rate according to a revised staging system can be predicted in these patients, but the response assessment needs to be further evaluated in larger studies [23, 24]. N Latex FLC is fairly new to the market and not all diseases have been specifically studied with these assays. More studies are needed to demonstrate the clinical performance of the tests.

The Seralite® dual κ and λ assay

Seralite® (Abingdon Health, York, UK) is a rapid and portable diagnostic device enabling the simultaneous quantitative measurement of serum κ and λFLC. Seralite® generates FLC results in a rapid timeframe and is designed to accelerate clinical decision-making near the patient. Using highly-specific and extensively validated mouse anti-human κ and anti-human λ mAbs [2], Seralite® is the first available FLC immunoassay to use a competitive inhibition format to prevent against false negatives caused by antigen-excess, a feature of non-competitive FLC assays that arises as a result of high FLC levels in patients with plasma cell dyscrasias.

Assay principle

Serum is mixed gently with sample application buffer provided with the Seralite® kit. As illustrated in Figure 3, an aliquot is then added to the Seralite® device, where it initially flows through a sample pad, and then onto a conjugate release pad containing gold-nanoparticle labeled anti-κ and anti-λ mAbs. These mAbs rehydrate, and together with the diluted sample, flow across a test membrane. At the end of the test membrane are two test lines, which contain purified κFLC or λFLC. The flow of sample and gold-labeled mAbs is allowed to continue for 10 min during which time any FLC in the sample will bind to its respective gold-labeled mAb; the higher the concentration of FLC in the sample, the less gold-labeled mAb will bind to the test line, and vice versa. The line intensity of the binding is determined by the Seralite® reader which also times the incubation, calculates the concentration of FLCs in the sample (from a stored calibration curve) and calculates the FLC ratio. The Seralite® initial calibration curve for κ and λFLC is 2.5–200 mg/L. If the FLC level in a sample is above 200 mg/L, a further sample dilution of one in 20 extends the upper range of the assay to 4000 mg/L This broad Seralite® calibration range, combined with the elimination of antigen excess, reduces the need for frequent sample dilution and is designed to improve laboratory workflow. The calibrators used for Seralite® are traceable to an original calibrator, which was tested on multiple batches of Freelite®, as there is no recognized international standard for FLC.

FLC antibody specificity

Over the past 20 years or so, numerous attempts have been made to generate anti-FLC mAbs that may be suitable for use in FLC immunoassays [3–5, 35]. To be efficacious, anti-FLC mAbs must identify epitopes that are exposed on FLC but hidden on LCs bound to heavy chains; thus eliminating mAb cross-reactivity with whole

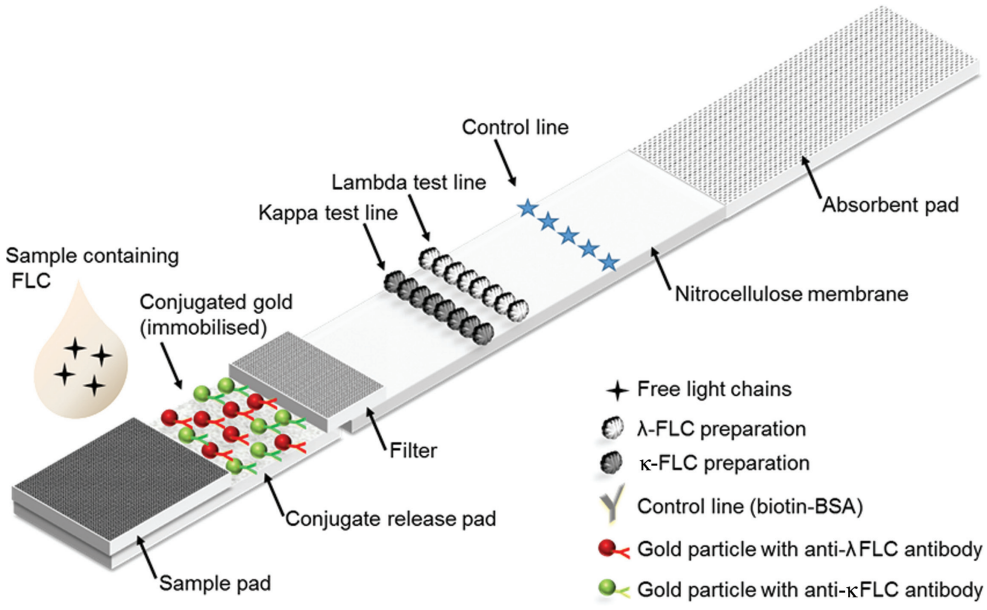

Figure 3: Schematic view of a single Seralite® device. Refer to text for assay principle.

immunoglobulin, a feature of polyclonal antibody based assays [35]. Crucially, the mAbs must detect each unique FLC secreted from a diverse range of plasma cells from all patients. This mission is made more difficult by the limited number of constant domain epitopes available on FLCs, and by the polymerisation of FLCs, particularly λ, which thus reduces the availability of mAb binding sites. As of yet, suitable mAbs that meet these criteria have not been met as these mAbs either: failed to identify monoclonal FLCs in patient samples, or were not validated against thousands of samples containing a wide variety of different monoclonal proteins [35, 36].

Seralite® utilizes anti-FLC mAbs which have been extensively validated against thousands of patient samples, firstly in a multi-plex bead array assay [2], and more recently in Seralite®. Initially, the mAbs were shown to successfully detect monoclonal FLC – where present and identifiable by the gold standard of urine-IFE, in 13,090 consecutive urine samples. The mAbs also identified monoclonal FLC in serum from 1000 consecutive clinical samples. Half of these 1000 samples contained monoclonal FLC, detected in paired urine samples by IFE, and it was found that the mAbs identified an abnormal FLC ratio in all of these samples and showed excellent diagnostic concordance with course of disease assessed by other laboratory tests of disease activity and clinical course documented in myeloma trial databases. Furthermore, the assay correlated excellently with Freelite® for the quantitation of normal polyclonal FLC in serum from healthy donors. Thus, the mAbs appear to be at least close to the ideal of detecting FLC from all plasma cell clones in all patients [2].

Analytical performance

The analytical performance of Seralite® is in submission in a separate publication and representative data is presented herein as an illustration of assay performance. Total imprecision has been assessed by the analysis of samples containing a range of FLC concentrations (5–74 mg/L). These were tested, as per the directions provided in the test kit, by three operators, on two Seralite® readers, using two different batches of test kits, on a total of 60 devices. There were no significant differences between readers, operators or kit lots and percentage CVs were between 9% and 12%. No significant interference was observed upon assessment of common interference agents, including cholesterol, triglyceride, bilirubin and hemoglobin added at final concentrations of 5.2, 11.3 mmol/L, 342 µmol/L and 2 g/L, respectively to samples with normal and elevated FLC levels. Together, these findings replicated Seralite® mAb performance in a prior report [2]. High levels of FLC in serum from patients with plasma cell dyscrasias have been shown to result in lower than expected FLC levels; a result of 'antigen excess' that affects direct turbidimetric and nephelometric immunoassays which also gives rise to non-linear dilutions [29, 32]. To prevent against

antigen excess, Seralite® utilizes a competitive inhibition format, which means that as FLC levels increase, available binding sites on the gold-labeled mAb become saturated and the mAb is inhibited from binding to the immobilized FLC antigen on the membrane; a false negative due to antigen excess is thus not possible [37]. To demonstrate elimination of antigen excess on Seralite®, Table 1 illustrates a number of case studies whereby antigen excess was evident on Freelite®, but not on Seralite®. Given the broad extremes exhibited by FLCs in the general population – ranging from below 1–2 mg/L in immune suppression to over 10 g/L in multiple myeloma – this is an important technical development that will ensure protection against antigen excess.

Assay performance in disease

Seralite® correlates with Freelite® in samples from healthy donors (Figure 4A–C), (unpublished data) where both assays exhibit similar quantitation of absolute polyclonal κ and λFLC, and FLC ratio. The results are significantly correlated (κFLC, $p<0.001$; λFLC, $p<0.001$; FLC ratio, $p=0.007$). Unlike Freelite®, Seralite® satisfactorily identifies FLC in patients with low levels of uninvolved FLC (Figure 4, panels D and E), and does not exhibit the 'gaps' between ~2 mg/L and ~7 mg/L that are seen in the lower end of the Freelite® assay range [2]. This feature of Freelite® must be taken into account when monitoring patients with plasma cell dyscrasias; an 'artefactual' shift from 7 mg/L to 1 mg/L in uninvolved Freelite® FLC level will cause an 'artefactual' seven-fold change in FLC ratio. Illustrated in the panel D and E of Figure 4 are uninvolved FLC in 247 myeloma patients at disease presentation; the Freelite® 'gaps' below 7 mg/L are clearly identifiable. In clinical practice, the FLC difference (involved FLC minus uninvolved FLC) is used to monitor response to therapy and monitor for relapse. Illustrated in panel F of Figure 4 is the FLC difference calculated for 114 patients at myeloma presentation; all patients presented with a FLC paraprotein visible in serum-IFE or in a paired urine sample by IFE. It can be seen that Seralite® and Freelite® exhibited diagnostic concordance, but quantitative estimation of monoclonal FLCs from neoplastic plasma cells was often divergent. Thus, in order to compare FLC levels between two samples from the same patient, it is important that same assay method is used; as is the case for monitoring whole paraprotein levels.

In a separate study, larger cohorts of myeloma patients (n=476) with light chain only myeloma, non-secretory myeloma, and intact immunoglobulin myeloma (IgA, IgG, IgD) were monitored through disease presentation, response to therapy, remission and relapse [38]. Seralite® provided excellent diagnostic concordance with Freelite® in these patient groups [38]. Seralite® clinical validation was extended to assess serial serum FLC measurements in patients who presented with IgA or IgG myeloma, who developed a relapse characterized by an increase in FLC (Δ>100 mg/L and >25%) without a corresponding increase in the heavy chain level, a phenomenon termed serum FLC escape (LCE escape) [39]. Seralite® identified an increase in the involved FLC and abnormal FLC ratio in all of these patients at LCE escape, and again demonstrated excellent diagnostic concordance with the Freelite® assay for κ and λFLC levels [39]. The clinical utility of Seralite® has also been demonstrated for diagnosing myeloma kidney in 288 patients with acute kidney injury (biopsy confirmed and all glomerular filtration rates <15 mL/min). Seventy-eight of these patients had a confirmed myeloma diagnosis and all were identified as having elevated involved FLC and an abnormal FLC ratio on Seralite®; Seralite® had excellent diagnostic concordance with Freelite® in all serum samples tested [40].

Taken together, these retrospective studies demonstrate that Seralite® is effective in diagnosing and

Table 1: Results from five samples that had antigen excess with Freelite® assay.

	Freelite®, mg/L		Seralite®, mg/L		Freelite®, mg/L		Seralite®, mg/L	
Sample	κ (initial dilution)	κ (final result)	κ (initial dilution)	κ (final result)	λ (initial dilution)	λ (final result)	λ (initial dilution)	λ (final result)
A	0.77	664.00	78.8	78.8	6.35	6.35	<2.5	<2.5
B	6.51	6.51	<2.5	<2.5	15.63	1772.00	>200	39,480.00
C	22.09	5435.00	>200	4960.00	10.77	10.77	6.7	6.7
D	37.64	31,594.00	>200	22,320.00	8.66	8.66	3.4	3.4
E	1.3	14,723	>200	32,160	5.34	0.85	<2.5	<2.5

Initial results with Seralite® and Freelite® assays are shown. Samples were then diluted at a higher dilution as recommended with the Freelite® assay when antigen excess is suspected.

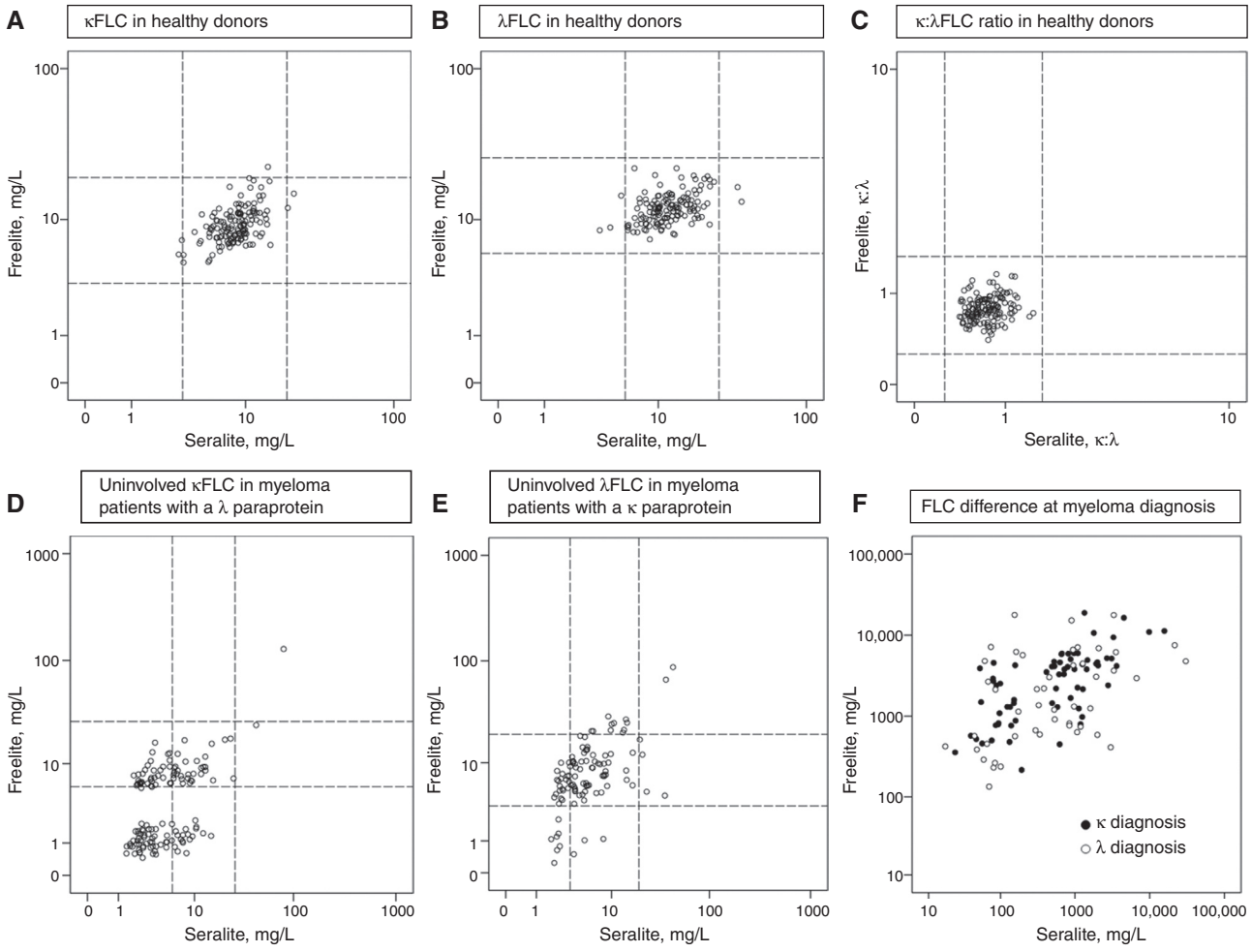

Figure 4: Comparison between Seralite® and Freelite® assays.
Plots A, B and C show comparative data for κFLC, λFLC and FLC ratio in healthy donors (n=144). Plot D illustrates uninvolved κFLC in myeloma patients with a λ paraprotein (n=109). Plot E illustrates uninvolved λFLC in myeloma patients with a κ paraprotein (n=138). Plot F shows comparative results for FLC difference (involved minus uninvolved), in 114 myeloma patients at diagnosis; 67 patients had κFLC in serum-IFE, and 47 patients had λFLC in serum-IFE. Dotted lines represent Freelite reference range for normal healthy donors.

monitoring plasma cell dyscrasias. The aforementioned studies have been presented at recent hematology meetings and, are in submission for publication elsewhere. Future independent studies will address the prospective utility of Seralite® in clinical practice. In conclusion, Seralite® is a rapid and portable diagnostic device that facilitates near-patient quantitation of FLC in serum. Using highly-specific and extensively validated anti-κ and anti-λ mAbs [2], Seralite® is designed to accelerate clinical decision-making and is the first available FLC immunoassay to use a competitive inhibition format to prevent against antigen-excess. Generation of rapid FLC results may empower clinicians to establish disease status and make informed treatment decisions more quickly and efficiently.

Conclusions

FLC measurements play a pivotal role in the screening, monitoring and prediction of progression of disease for patients with plasma cell dyscrasias. The great number of publications on FLC measurements demonstrates that patient management greatly benefits from the use of FLC assays. The introduction of the monoclonal-based FLC assays has opened new perspectives for the clinical laboratory and allows rapid testing in the clinic. It has demonstrated the analytical pro- and cons of the old and the new assays, but it also highlighted that the monoclonal κ and λLC proteins come in many forms and the same FLC may be measured at different concentrations depending on the assay. Although the correlations and concordance between the assays are

good between the polyclonal and monoclonal-based FLC assays, the results are not interchangeable, as is well established for different methods to detect whole M-proteins. Indeed, during the development of the Seralite® assays, it became apparent that the measured concentrations of FLC in serum depend on the antibodies used for the assays [2]. Crucially, however, the monoclonal-based assays herein provide similar reaction patterns to the polyclonal-based assays, and high levels of involved monoclonal FLC are identified at disease presentation, show a reduction during successful disease therapy, and an increase at disease progression. Thus, an adequate period of parallel testing should enable the continued accurate follow up of the patient if changing from one test method to another. Benefits of using mAbs assays include that users evade lot-to-lot variations as seen – even with successive batches of polyclonal antibodies – on Freelite®. Indeed, within the collection of Freelite® assays, customers notice differences in outcome in EQAS studies when the assays are performed on different systems [20]. This shows that – in a similar manner to whole M-protein assays – results for different FLC assays on different clinical analyzers are not interchangeable. At the same time, the differences between the methods demonstrate how difficult standardization of the FLC assays will be.

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